

THE INFLUENCE OF MONTELUKAST ON THE ACTIVITY OF THE AUTONOMIC NERVOUS SYSTEM ESTIMATED BY HEART RATE VARIABILITY IN EXPERIMENTAL PARTIAL BLADDER OUTLET OBSTRUCTION IN RATS

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Abstract: Due to their paracrine action, leukotrienes released from the urothelium are involved in control of the bladder function. Anti-leukotriene agents appear to exert an ameliorating effect in bladder overactivity. It is unknown, whether their possible, modulatory impact on the autonomic nervous system (ANS) activity may also contribute to the potentially beneficial effect of those compounds. Therefore, our aim was to indirectly estimate the ANS function using the heart rate variability (HRV) study in rats with experimental partial bladder outlet obstruction (PBOO), reflecting human benign prostatic hyperplasia (BPH), treated with leukotriene receptor antagonist - montelukast (MLKT). Twenty rats with surgically induced PBOO lasting for 14 days, divided into two groups: group 1 (10 control subjects) and group 2 (10 MLKT-treated rats; 2 mg/rat/day) were subjected to HRV recordings, preceded by daily urine collection and a subsequent cystectomy with histopathological evaluation of collected bladders. Standard HRV time and spectral parameters were calculated. MLKT-treated animals demonstrated an increase in power of non-normalized LF (low frequency) and HF (high frequency) components with no change of the total HRV power. Moreover, an increase and decrease in normalized nLF and nHF, respectively, were assessed in those animals compared to the control. Additionally, a decrease in daily diuresis measurement was demonstrated in MLKT-treated animals. Montelukast treatment resulted in the functional ANS status re-arrangement, with sympathetic overdrive and parasympathetic withdrawal. Those changes may contribute to alleviation of bladder overactivity symptoms, independently on leukotriene receptors blockade.

Keywords: partial bladder outlet obstruction, benign prostatic hyperplasia, bladder overactivity, leukotriene, montelukast

Abbreviations: ANS – autonomic nervous system, BPH – benign prostatic hyperplasia, BW – bladder wet weight, COX – cyclooxygenase, CP – cyclophosphamide, CP-HC – cyclophosphamide-induced hemorrhagic cystitis, HF – high frequency (HRV spectral, non-normalized component), HRV – heart rate variability, LF – low frequency (HRV spectral, non-normalized component), LOX – lipoxygenase, LUTS – lower urinary tract symptoms, MLKT – montelukast, MLX – meloxicam, nHF – normalized high frequency (HRV spectral, normalized component), nLF – normalized low frequency (HRV spectral, normalized component), NN – an interval between two subsequent dominant, sinus R waves in ECG recording, NSAIDs – non steroidal antiinflammatory drugs, OAB – overactive bladder, PBOO – partial bladder outlet obstruction, PRX – piroxicam, rMSSD – the square root of the mean squared difference of successive NN intervals, SDNN – standard deviation of all normal-normal (N-N) beats, TP – total power (HRV spectral, non-normalized component), VLF – very low frequency (HRV spectral, non-normalized component)

Benign prostate hyperplasia (BPH) is one of the most common urological disorders diagnosed in aging male population. BPH is an overgrowth of both epithelial and smooth muscle cells within the prostatic transition zone and periurethral area (1, 2). That results in hypertrophic prostate tissue compres-

sion of the proximal urethra and in progressive increase of the lower urinary tract pressure, finally leading to an impaired urinary flow. The incidence of BPH is closely associated with age – it is a rare condition in men under the age of 40, but nearly 50% of 50+ men develop BPH symptoms, and it is

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diagnosed in nearly 80% of elderly patients (age 80 and over) (3, 4). This pathological condition results in – so called – lower urinary tract symptoms (LUTS), that may be divided into both obstructive (weak urine stream, straining, prolonged and incomplete voiding) and irritative (increased urinary frequency, urgency, urge incontinence, nocturia) ones (1, 5). Thus, BPH is regarded to be a clinical condition characterized by secondary bladder overactivity, similarly to primary, idiopathic overactive bladder syndrome (OAB), but induced by organic, sub-vesical obstruction and bladder hydrostatic pressure overload (1, 6).

Despite its high prevalence, the pathogenesis of BPH is only partially known. The current pathophysiological BPH description is focused on the hormonal deregulation with elevated dihydrotestosterone prostatic amount and enhanced secretion of the fibroblast growth factor (FGF) impairing processes of apoptosis. Those disturbances promote overgrowth of the prostate tissue. Much attention is also drawn to the prostatic production of highly tissue-damaging free radicals in response to local hypoxia (7). However, the chronic prostatic inflammation seems to be the key factor in BPH development. The evidence of prostatitis was found in approximately 90% of histological examination of samples collected during the transurethral resection of the prostate (8, 9). Moreover, the degree of inflammation was found to be correlated with the prostate volume and weight in patients with BPH (10). The origin of chronic prostate inflammation is ambiguous and some factors, such as: bacterial infections, viruses, sexually transmitted organisms, dietary factors, hormones, autoimmune response and urinary reflux are considered to both initiate and maintain the inflammatory response (8). That process disrupts the homeostasis between cellular death and proliferation, with final predominance of hyperplasia and reduction of the apoptotic process (1, 2). During BPH, an inflammatory reaction is also present in the urinary bladder, being co-responsible for irritative LUTS. A special role of arachidonic acid derivatives – prostaglandins and leukotrienes is currently emphasized in pathogenesis of inflammation, among various inflammatory mediators. Both isoforms of cyclooxygenases (COX) 1 and 2 (constitutive and inflammatory-inducible one, respectively) have been found in the prostate gland (11) and there are evidences that COX-2 mediated PG cause the increased expression of anti-apoptotic protein Bcl-2 (12).

Lipoxygenase (LOX) isoforms are also overexpressed in prostate tissues, and they have been found

in prostate cancer cells (13). Independently, most of inflammatory cells (e.g., polymorphonuclear leukocytes or macrophages) have also an ability to synthesize leukotrienes that are regarded to be strong chemoattractant factors, also promoting adhesion to the inflamed and damaged tissue (2).

The urinary bladder contains also abundant eicosanoids, commonly regarded to be important paracrine factors involved in the bladder control (14).

Summing up, both prostaglandins and leukotrienes, as synthesized lipid inflammatory mediators, seem to be an attractive pharmacological target, and anti-leukotriene agents appear to exert the ameliorating effect in BPH and other conditions associated with bladder overactivity. That hypothesis was confirmed by Altavilla et al. (2), who demonstrated that the use of flavocoxid in rats with testosterone-induced experimental BPH resulted in substantial reduction of the prostate weight and hypertrophy along with blunted production of prostaglandin E_2 and leukotriene B_4 , with apoptosis stimulation and inhibited growth factor expression. Since the flavocoxid is a dual inhibitor of both cyclooxygenase and lipoxygenase, their results suggest that modulation of eicosanoids production may be a rational approach to reduce LUTS in the course of BPH. However, it is uncertain whether the influence of eicosanoids on the autonomic nervous system (ANS) function may be also considered as an additional pharmacodynamics feature of novel, eicosanoid-targeted agents.

The autonomic nervous system function may be indirectly estimated using the heart rate variability (HRV) method based on the measurement of successive R-R (also called NN; normal-normal) intervals variation in ECG recordings in terms of temporal and spectral (frequency) analysis (15). BPH is relatively easily reproduced in an animal model (by surgical formation of a partial bladder outlet obstruction; PBOO) (16).

Thus, the aim of our study was to estimate the ANS function using the heart rate variability (HRV) in an experimental BPH model of partial bladder outlet obstruction (PBOO) in rats treated with the leukotriene receptor antagonist – montelukast (MLKT). Furthermore, we have also evaluated whether the expected, modulating impact of MLKT on the autonomic activity may contribute to the beneficial, desired effect of this compound. Hence, we have attempted to determine if due to its modulatory effect on the ANS activity, in addition to the anti-inflammatory effect, MLKT may be considered a potential drug used for alleviation of bladder dysfunction.

EXPERIMENTAL

Ethical issues

The medical experiment described in this paper was approved by the appropriate local ethics committee responsible for carrying out experiments on animals in Kraków.

Animals used for the experiment and a general plan of the study

The experiment involved 20 eight-week-old albino Wistar rats with an average body weight of 194.4 ± 7.9 g, obtained from the central animal house of the Faculty of Pharmacy UJCM. Upon arrival to the local animal house of the Department of Pathophysiology, during the first week, the animals acclimated to new living conditions. During that period, animals were housed in standard conditions, five individuals per cage, with unlimited access to water and food (Labofeed, Kcynia). The experiment commenced on the second week with measurement of daily water intake by placement of intact rats in single metabolic cages. The partial bladder outlet obstruction (PBOO) surgery was performed on the following day in all animals. One subject fell during the surgery and another one on the second day of the postoperative period. Hence, 18 rats were finally subjected to further studies. The post-surgical recovery period lasted two days. On the third day, the daily intake of water was re-measured, and animals were randomly allocated to two groups: control (group 1; $n = 9$) or test group of animals treated *per os* with the leukotriene receptor antagonist - montelukast (group 2; $n = 9$).

Beginning on the fourth day after the PBOO procedure, the animals in the group 2 received MLKT. All animals demonstrated normal activity with no signs of postoperative complications.

On days 4 to 14 after the PBOO induction, both control and test animals were kept in individual cages, with limited access to water (in the volume corresponding to the individual, average daily water intake, previously determined with repeated measurements) and with unlimited access to food. Animals in the group 2 received montelukast (obtained from commercial preparation - montelukast, Sandoz, sachets with 500 mg granulate containing 4 mg of active substance) dissolved in the measured volume of water. The montelukast dose of 2 mg/rat/day was applied, taking into account the average body weight of 217.0 ± 11.5 g of animals in the group 2 at the 15th day of the experiment, corresponding to 9.2 mg/kg body weight/day. The dose applied by us was consistent with the

MLKT amount reported by other investigators - 10 mg/kg *p.o.* (17, 18), and also corresponds to the dose administered intraperitoneally - 10-30 mg/kg *i.p.* (19, 20). On the penultimate day of the experiment, all animals were once again placed in single metabolic cages with maintained access to the previously calculated volume water, and MLKT in group 2, to finally assess final body weight and the daily urine excretion.

On the 15th day of the experiment, ECG recordings with subsequent HRV analysis were carried out for all animals to obtain standard time and spectral parameters. The procedure was completed under general pentobarbital anesthesia. Finally, after the ECG recording, animals were sacrificed with a lethal dose of pentobarbital and cystectomy was performed to determine the bladder wet weight (BWW) and to obtain bladder tissue for the subsequent histopathological assessment.

The PBOO surgery

The procedure of partial ligation of the proximal urethra (partial bladder outlet obstruction) was performed under general pentobarbital anesthesia (moribital, 60 mg/kg body weight (b.w.); calculated for the pentobarbital sodium). After making an incision in the midline of the body (in the umbilical ligament plan), an urinary bladder was unveiled, and following catheterization (epidural catheter, 1 mm diameter) the proximal section of the urethra was ligated (Dermalon 4-0). Then, catheter was removed leaving the urethra partially occluded and integuments were sutured (Dermalon 4-0). During first two post-surgical days, neomycin and oxytetracycline sprays were applied once a day to the postoperative wound.

HRV recordings

Twenty minute-long ECG records under general, pentobarbital anesthesia (moribital at a dose of 60 mg/kg b.w. calculated for the pentobarbital sodium) were performed for all study animals. The ECG signal, as a starting point for the subsequent HRV analysis, was acquired using pediatric electrodes Ag/AgCl (EK-S30 Sorimex PSG) and the Polygram system ADInstruments hardware and software, after prior hair removal and application of both abrasion paste and covering the skin with a standard ECG gel. Based on the obtained ECG recordings, standard time parameters: mean, max. and min. NN intervals, standard deviation of all NN intervals (SDNN), root mean square of successive differences (rMSSD) – all in [ms], and the mean heart rate HR [1/min] as well as spectral (frequency) ones: total HRV spec-

trum power (TP), power of the very low frequency component (VLF), power of the low frequency component (LF) and power of the high frequency component (HF) – all in [ms²] - were calculated (15, 21–25). We performed also the HRV spectrum normalization. It is a re-creation of the initially obtained spectrum to the secondary one with the VLF bypassing. Although the VLF power is a primary contributor to the total power (TP) development, by indicating the activity of slow thermoregulatory mechanisms, chemoreceptor stimulations or various neuroendocrine mechanisms (e.g., the renin-angiotensin-aldosterone system), it also includes some unknown, not fully understood, autonomic mechanisms. Therefore, the primary obtained HRV spectrum is usually subjected to re-calculation for share of relevant components (LF or HF) in total HRV power, excluding the VLF component power. That procedure allows determination of two normalized nLF and nHF parameters - expressed in normalized units ([n.u.]) (15, 21–25).

The spectrum bands for respective components was set as: 0.18 < VLF < 0.28 < LF < 0.78 < HF < 3, consistently with values applied by other researchers: Aubert et al. (26) (0.19 < LF < 0.74 < HF < 2.5), and Goncalves et al. (27) (0.10 < LF < 1.0 < HF < 3.0). Results were presented as the mean values ± SD.

Cystectomy and histopathological evaluation

In all study rats, the bladders were collected once the HRV recordings had been taken and following administration of a lethal sodium pentobar-

bital dose (160 mg/rat; calculated for pentobarbital sodium), to ascertain the changes in bladder wet weight (BWW) and its percentage weight in relation to the final body weight. Bladders were cut off with the proximal, ligated urethra. They were gently drained off and immediately weighted. According to the literature, cystitis may be evaluated not only by determination of changes in macroscopic and microscopic analysis but also by evaluation of the bladder wet weight. Thus, BWW is regarded to be an indirect marker of cystitis and bladder dysfunction (28, 29). When the BWW value was determined, collected bladders were placed in 4% formalin + PBS solutions for further histopathological assessment. The finally prepared microscopic sections were H + S stained to enable the histological evaluation of intensification of the inflammatory process.

Statistical analysis

Due to absence of normal distribution of obtained HRV parameters, statistical analysis was performed after their expression as natural logarithms. Parametric Student’s *t*-test with α = 0.05 was applied to evaluate the statistical significance. The conclusions of HRV results and statistical inference are given in Table 2 and Figure 1.

RESULT

The final body weight of study animals

In both study groups a trend of the body weight increase was observed throughout the experiment (15 days), compared to the value measured in the

Table 1. The characteristic of studied animals with PBOO model.

	Group 1 control PBOO rats	Group 2 PBOO rats + MLKT	Statistics
Starting body weight [g]	198.4 ± 7.9		
Final body weight on 14 th day [g]	203.2 ± 8.6	217.0 ± 11.5	NS
Average daily water intake [mL]	20.7 ± 3.5	24.4 ± 6.3	NS
Bladder wet weight [mg]	145.0 ± 19.1	150.6 ± 40.9	NS
Bladder wet weight related to final body weight [%]	0.058 ± 0.033	0.069 ± 0.017	NS
24-h urine excretion on 14 th day [mL]	6.9 ± 1.0	5.3 ± 1.5	p = 0.02

PBOO – partial bladder outlet obstruction, MLKT – montelukast, NS – non significant.

Table 2. Time- and spectral-domain HRV analysis parameters obtained from 20-min ECG recordings in rats with PBOO model.

	PBOO rats control	PBOO rats treated with MLKT	Statistics (for ln values)
TIME-DOMAIN HRV ANALYSIS			
mean NN [ms]	173.03 ± 11.88	174.38 ± 7.29	NS
max NN [ms]	188.33 ± 1.71	188.43 ± 9.82	NS
min NN [ms]	152.64 ± 16.72	141.66 ± 4.30	NS
range [ms]	35.76 ± 16.29	46.88 ± 4.05	p = 0.04
average HR [bpm]	348.29 ± 25.26	345.28 ± 12.67	NS
SDNN [ms]	7.43 ± 3.96	9.66 ± 2.79	NS
rMSSD	6.92 ± 8.26	9.24 ± 7.64	NS
SPECTRAL-DOMAIN HRV ANALYSIS			
TP [ms ²]	23.16 ± 32.56	28.74 ± 16.79	NS
VLF [ms ²]	20.87 ± 30.33	23.70 ± 14.96	NS
LF [ms ²]	1.31 ± 1.79	2.71 ± 1.73	p = 0.02
HF [ms ²]	0.98 ± 0.96	2.33 ± 1.78	p = 0.05

PBOO – partial bladder outlet obstruction, MLKT – montelukast, HRV – heart rate variability, mean NN – mean normal-normal interval [ms], max NN – maximal normal-normal interval [ms], min NN – minimal normal-normal interval [ms], range – mean difference between max and min intervals [ms], average HR – average heart rate [bpm – 1/min], SDNN – total standard deviation of all normal-normal intervals [ms], rMSSD – root mean square of successive differences, TP – total power of HRV spectrum, VLF – power of very low frequency component in the HRV spectrum, LF – power of low frequency component in the HRV spectrum, HF – power of high frequency component in the HRV spectrum, nLF – normalized LF, nHF – normalized HF, NS – non significant.

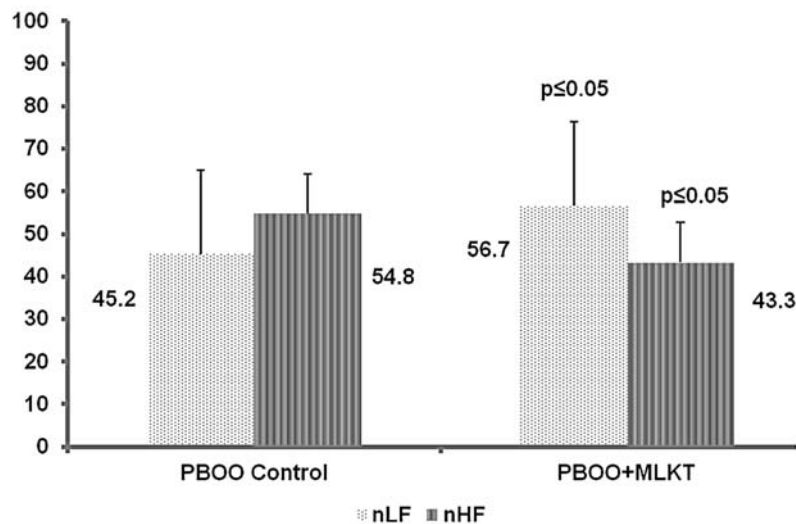


Figure 1. Normalized spectral components values [n.u.] in studied animals with PBOO mode

beginning of the study. The final body weight in both control and MLKT-treated animals achieved similar values with no significant difference. Detailed results are presented in Table 1.

Average daily water intake and final 24-hour urine collection

The evaluated average daily water intake maintained during the following days of the experiment

was similar in both studied groups. Thus, on days 4 to 14 each rat was supplied with an individually determined water volume with (group 2) or without (group 1) the addition of MLKT. Finally, on the day 15 a 24-h daily urine collection procedure indicated a lower daily urine volume in MLKT-treated animals compared to the value obtained in control rats. The difference was statistically significant. The results are presented in Table 1.

The bladder wet weight and histological assessment of collected bladders

Neither the bladder wet weight nor the calculated percentage of the bladder wet weight related to the final body weight differed significantly in study animals.

However, BWV value determined in parallel in intact animals (5 individuals) during our experiment was 120.1 ± 6.3 mg. That value significantly differed from the BWV results demonstrated in both control PBOO animals (145.0 ± 19.1 mg; $p \leq 0.05$), as well as in PBOO animals treated with montelukast (150.6 ± 40.9 mg; $p \leq 0.05$).

The higher BWV observed in PBOO rats can be considered as an indirect proof supporting the thesis of bladder tissue remodeling resulting from bladder adaptation to increased resistance due to the bladder outlet obstruction. Therefore, those results also confirm the efficacy of the performed PBOO surgery.

The detailed results relating to both study groups are presented in Table 1.

The time-domain HRV analysis

All time-domain HRV parameters, with exception of the range of NN intervals, were comparable in both groups and did not significantly differ between groups. Animals treated with MLKT demonstrated a globally increasing trend of both SDNN and rMSSD, although with no evidence of statistical significance.

The results of the time-domain HRV parameters with statistical inference are presented in Table 2.

The spectral HRV analysis

The performed spectral (frequency) HRV analysis revealed some statistically significant intergroup differences related to the power of the primary, non-normalized spectral components in the range of low (LF) and high (HF) frequencies. Higher values were observed in MLKT-treated rats (group 2). The observed changes concerning LF and HF components were not accompanied by the parallel change in the total power (TP) HRV spectrum. In addition, the non-normalized very low frequency

(VLF) component power also did not significantly differ between both analyzed groups.

Results of the spectral HRV analysis and values of non-normalized HRV components with statistical inference are presented in Table 2.

Another differences between studied groups were in values of nLF and nHF, which were statistically significantly higher and lower, respectively, in montelukast-treated rats compared to control animals. Those differences are shown in the Figure 1.

Histological analysis

A diffuse, minor and chronic inflammation was observed in stroma of the bladder mucosa in specimens of bladders collected from rats with PBOO receiving no montelukast. Focal signs of squamous epithelial metaplasia were found in some cases.

In all assessed specimens obtained from animals treated with montelukast (the PBOO + MLKT group) the histological presentation was normal.

DISCUSSION

The main findings of our experiment may be summarized as follows:

1. Rats with the experimental partial bladder outlet obstruction, treated with montelukast, were characterized by different autonomic nervous system activity compared to untreated animals within the same model. The 10-day long treatment with montelukast (leukotriene receptor antagonist) resulted in increased power of non-normalized LF and HF components with the total HRV power remaining unchanged. Moreover, the additional evidence of functional ANS rearrangement in MLKT-treated individuals was also evidenced by statistically significant nLF increase and nHF decrease in those animals, compared to control.

2. Rats with the experimental partial bladder outlet obstruction, treated with montelukast, after the 10-day long treatment excreted less urine daily compared to untreated animals and the difference was statistically significant.

3. In histopathological assessment, control group demonstrated some minor inflammatory changes, and MLKT-treated animals presented regular histological structure.

In accordance with generally accepted HRV guidelines (15), the power of respective spectral components as well as changes in time-domain HRV parameters reflect the activity of sympathetic and/or parasympathetic part of the ANS.

SDNN and TP correlate with the global autonomic tension whereas the range of NN variation,

rMSSD and HF are regarded to be markers of selected parasympathetic activity. LF is a spectral component frequency of which reflects various phenomena mediated by both sympathetic and parasympathetic parts of the ANS (15, 21–25).

Therefore, taking into account the observed HF increase in HRV spectrum of MLKT-treated animals, the hypothesis of parasympathetic overactivity in the conditions of antileukotriene blockade may be considered. However, it is ambiguous considering the simultaneous increase of the LF component in that group of animals. The observation may be also interpreted as a result of augmented sympathetic activity. Furthermore, analyzing the main time-domain HRV parameter associated with the parasympathetic tension (rMSSD), no significant change was observed. It is therefore obvious that the reasoning concerning the assessment of autonomic nervous system activity based on the results of both time and non-normalized spectral HRV analysis is uncertain. Hence, the next step of our analysis was based on the assessment of normalized HRV spectrum parameters, thought to reflect the selective parasympathetic (nHF) and sympathetic (nLF) tension.

Taking these premises into account, and in connection with equivocal results of the basic spectral analysis, we concluded that clear and statistically significant sympathetic predominance (nLF increase) along with parasympathetic withdrawal (nHF decrease) was observed in MLKT-treated animals. It could be then expected that experimental PBOO in preserving leukotriene action might be related to the reverse ANS functioning and leukotrienes would be considered as the agents contributing to a high parasympathetic and low sympathetic tension.

Moreover, the leukotriene action blockade, resulting in the abovementioned rearrangement of functional ANS status, with the observed sympathetic overdrive, could contribute to alleviation of bladder overactivity symptoms, that was consistent with observed changes in our daily diuresis measurements following a 10-day long MLKT administration.

Our results support also the overall concept concerning the role of arachidonic acid derivatives in the bladder pathophysiology. There is evidence that bladder overactivity results from abnormal bladder paracrine activity, associated with release of prostanoids of urothelial origin. Those agents are thought to influence the bladder function, both by their inflammatory action directed to the detrusor, and a secondary involvement in neuronal control of

the bladder (30, 31). In general, according to the basic bladder neurophysiology, parasympathetic fibers and acetylcholine released from efferent terminals and affecting muscarinic receptors, are involved in bladder contraction and emptying, and their overstimulation may result in detrusor instability. On the contrary, sympathetic innervation and noradrenaline-mediated stimulation of adrenergic, β -3 bladder receptors contribute to detrusor relaxation and increase bladder compliance (30, 31). Moreover, a link between PGs and the muscarinic system has been described previously (32). Ikeda et al. (33) demonstrated also the inflammatory-facilitated afferent nerve activity *via* EP1 receptors in animal models. To sum up, stimulation of EP1 and EP3 receptors leads to contraction of urinary bladder smooth muscle cells, whereas stimulation of EP2 and EP4 receptors causes muscle relaxation (34).

Thus, arachidonic acid derivatives affecting autonomic fibers are important agents, co-responsible for bladder function control. In consequence, pharmacological agents abolishing prostanoid-induced bladder overactivity seem to be a future, potential therapeutic option in patients presenting LUTS. Among those agents, non-steroidal anti-inflammatory drugs (NSAIDs) are thought to be a new, promising therapeutic option, consistent with clinical observations of many researchers (35–38). NSAIDs seem to be a possible pharmacological choice in treatment of BPH patients due to their expected reduction in the severity of irritative LUTS, and to a lesser extent, of obstructive LUTS (39). Modulation of the EP1 and EP2 receptor activity (directly *via* EP1/EP2 antagonists or indirectly *via* COX inhibitors) could be also a therapeutic goal in urinary bladder dysfunction.

Moreover, NSAIDs are also consider to alleviate symptoms of bladder overactivity by autonomic activity modulation. In one of our previously published works (40) we evaluated the effect of piroxicam-induced (both in the low – 2 mg/kg b.w. and high – 10 mg/kg b.w. doses) prostaglandins synthesis block on activity of the ANS in bladder outlet obstruction. We revealed that piroxicam (PRX) in both doses produced a trend for reduction of value of all non-normalized components of HRV. The lower PRX dose caused an increased nHF value, and PRX administered in the dose of 10 mg/kg b.w. caused an increase of the nLF value.

In our other experiments we applied preferential COX-2 inhibitor - meloxicam (MLX; 5 mg/kg b.w.) to determine the effect of that compound on the ANS activity, in both PBOO (41) and CP-HC (42) models. We did not demonstrate any significant dif-

ferences in time and spectral HRV analysis in animals treated with MLX compared to the corresponding control groups. Therefore, these results suggest that COX-1 derived constitutive prostaglandins seem to exert neuromodulating properties and inhibition of prostaglandins synthesis with PRX administered at the dose of 10 mg/kg b.w. lead to functional reconstruction of ANS, with marked sympathetic predominance. That may contribute to reduction of the bladder contractile action and improvement of its compliance in the filling period, which was demonstrated by other authors in urodynamic tests for NSAIDs (40). Our findings are also further rationale for using NSAIDs in the treatment of bladder overactivity symptoms. However, the broad usage of NSAIDs in OAB-related disturbances treatment are still limited due to their serious side-effects when administrated systemically (*p.o.*) (39).

On a margin, our choice of PRX and MLX, belonging to NSAID subgroup constituting “oxi-cams” family, applied in our previous studies, resulted from the need for the using of active compounds with a slight difference in their detailed pharmacodynamics aspects, (considering their selectivity in relation to COX: non-specific vs. preferential, respectively) and preserving their maximal therapeutical and chemical structure similarity (expressing by the same main ATC code: M 01 A C).

We are aware of limitations of our experiment – our reasoning concerning the ANS function was based only on the indirect method of HRV and it has not been supported by any direct evidence, not even the plasma noradrenaline level measurements (as an indicative, laboratory marker of sympathetic activity).

In addition, conclusions regarding the role of leukotrienes in the pathogenesis of CP-induced cystitis were also made indirectly, based on the results obtained in terms of their receptor-blocking action. However, despite these limitations, our preliminary results suggest the necessity of further research, that should clarify the pathophysiological role of leukotrienes in BPH and other LUTS-related disturbances, as well as the therapeutic value of leukotriene-targeting antagonistic agents.

CONCLUSIONS

In the present paper we suggest that, in addition to prostaglandins, leukotrienes also play a modulatory role in autonomic control of the bladder, and the leukotriene antagonists also seem to be an effective therapeutical options in LUTS-associated disturbances.

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Conflict of interest

The authors declare that there is no conflict of interest in the authorship.

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